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Probiotic Treatment as a Potential Therapeutic Intervention for Hepatocellular Carcinoma: A Literature Review

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Abstract

Traditionally, liver cancer has been treated by surgical procedures and medications that eliminate cancer cells. Despite having a high success rate, these treatments have limitations that can cause cancer recurrence or contribute to failed treatment outcomes. The gut microbiota can potentially play a significant role in the prevention, initiation and advancement of liver cancer. Recently, dysbiosis in the gut microbiota has been found to implicate several gastrointestinal diseases, such as Colorectal Cancer (CRC). The proposed probiotic therapy can restore this dysbiosis by introducing beneficial bacteria in the form of probiotics into the gut microbiome. These probiotic bacteria could produce beneficial Short-Chain Fatty Acids (SCFAs), induce anti-inflammatory effects and prevent the release of toxic substances from the gut into the hepatic portal circulation. The rationality of using probiotics is outlined through current treatment applications that have successfully treated cancer among other chronic diseases. This paper discusses the promising activity of novel bacterial strains that could be utilized as probiotics to improve beneficial gut microflora for liver cancer treatment. Lastly, the paper discusses the future prospects of probiotics and their application in other liver diseases.

Keywords: Hepatocellular carcinoma; Colorectal cancer; Asymptomatic stage of cirrhosis; Programmed death receptor

Introduction

Hepatocellular Carcinoma (HCC) or primary liver cancer, is a globally prevalent disease that accounts for approximately 800,000 deaths yearly, making it the sixth most common cancer and the fourth most common cause of death in the world. Data extractions from previous studies suggest that the incidence and mortality rates of liver cancer could increase by over 55% by the year 2040. The majority of liver cancer cases are found to occur in developing countries at an incidence rate of 80%. Evidently, east and southeast Asia, along with central and western Africa, exhibit the highest incidences of liver cancer, whereas regions such as south central and western Asia, as well as northern and eastern Europe, present lower rates of this disease. In 2018, it was estimated that there were 841,000 cases (9.3 cases per 100,000 people/year) and 782,000 deaths (8.5 deaths per 100,000 people/year), accounting for the fourth leading cause of cancer mortality worldwide. Additionally, there is a clear gender disparity in liver cancer occurrence, with men being affected by this disease at a significantly higher rate than women, with estimates suggesting a prevalence between two to eight times higher [1].

The development of liver cancer stems from a multitude of factors that include birth defects, alcohol abuse, chronic viral infections such as hepatitis B and C, hemochromatosis and cirrhosis. It has been concluded that chronic liver disease and cirrhosis remain the most important risk factors in the development of HCC of which viral hepatitis and excessive alcohol consumption are the leading factors globally. Specifically, the Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) strains are the most common causes of HCC. The regional differences in liver cancer incidence reflect the variations in exposure to hepatitis viruses and environmental pathogens. For example, HBV infection accounts for about 60% of the total liver cancer in developing countries, whereas HCV infection accounts for about 33% of total liver cancer incidence. As for the prevention and treatment of HCC, more effective therapeutics are needed due to the limitations of the current treatments that will be discussed.

Given the direct association between the liver and the intestines through the hepatic portal circulation, numerous studies have insinuated a causal relationship between alterations in the gut microbiota and HCC progression. It has

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been recently reported that gut microbiota-derived products can restore these maladaptive alterations and modulate hepatic inflammation and immunity to reduce the rate of HCC progression. This encouraged the possibility of using a newly emerging field of cancer therapy, known as probiotics, to restore the HCC-associated dysbiosis in the gut microbiome. Our literature review aims to discuss the limitations of current liver cancer therapeutics and introduce existing and potential probiotic bacteria that could serve as a promising therapy for liver cancer.

Literature Review

The first step towards conducting the literature review was using a combination of search terms to find relevant articles on different databases. The search term 'probiotics or liver cancer or hepatocellular carcinoma or gut microbiome or microbiota or short-chain fatty acids or *Lactobacillus* or *Bifidobacterium* or *Faecalibacterium*' was used to conduct a search in Google Scholar, which were then added to an online screening tool called covidence. Initially, a total of 427 articles were added to covidence from Google Scholar. There were no duplicates. Next, inclusion and exclusion criteria were used to determine the relevancy of the remaining articles. The screening was completed independently by two reviewers with a third reviewer resolving any discrepancies. Any articles that were published before the year 2014, written in a language other than English or lacking scientific data (editorial, conference abstract, trial description) or irrelevant information were excluded. In the first round of screening, the two reviewers screened the abstracts of the articles to exclude any non-eligible articles. This step resulted in the exclusion of 242 articles, leaving 185 articles for further screening. In the second step, the full-body texts of the remaining articles were screened to determine their eligibility in terms of the presence of relevant information. After the thorough screening, only 63 articles were left that were used to extract information and data needed to conduct this review. This was followed by the Currency, Relevance, Authority, Accuracy and Purpose (CRAAP) test which allowed us to evaluate, assess and eliminate bias from the reviewed article with the help of a list of questions. For quality check, the Critical Appraisal Skills Programme (CASP) checklist was used to assess the trustworthiness, relevance and reliability of the published paper [2].

Current treatments for HCC

Multiple treatment options are available for HCC. The applicability of these treatments depends on factors such as the tumor stage, patient performance status and liver function reserve, which need to be determined using a multidisciplinary approach. For instance, patients diagnosed with advanced stages of HCC would more likely be administered targeted drug therapy rather than surgical resection. In this section, current therapies that are commonly used to treat HCC and their limitations will be discussed [3].

Surgical resection: Surgical resection refers to the surgical removal of a piece of the liver and can be referred to as partial hepatectomy as well. Generally, surgical resection is considered for patients with non-metastatic tumors, normal underlying liver function or compensated cirrhosis (asymptomatic stage of cirrhosis) and without evidence of portal hypertension. In general, patients with HCC in early stages would be eligible for this treatment. Overall, undergoing surgical resection can achieve a five-year survival rate of about 70%, given that the patients have satisfied the criteria for taking this treatment option. However, poor prognosis is associated with this treatment as the recurrence rate remains high. Recurrence is usually correlated with the appearance of Microscopic Vascular Invasion (MVI), which is defined as the presence of malignant cells lining the vascular cavities of endothelial cells or portal and hepatic venous systems. To lower the risk of recurrence, biomarkers in patients, such as gene signatures or molecular biomarkers, are observed prior to surgery to help predict the probability of recurrence post-surgery, improving the selection of candidates for surgical resection with lower risk of recurrence.

Liver transplantation: Liver transplantation is regarded as the most ideal treatment option for patients with early-stage tumors. This procedure replaces the patient's liver with a healthy liver from the donor. With the removal of any underlying liver diseases (which are a major risk factor for new tumor development), this treatment tends to grant patients the highest chance of being cured, with a 5-year survival rate exceeding 70%. Careful selection of the candidates who could receive the transplantation and donors of the liver is critical to success. One of the common ways which physicians adopt to determine a patient's eligibility for the surgery is the Milan criteria, which states that patients who receive liver transplantations should have a single tumor less than 5 cm in diameter or 3 tumors that are all greater than 3 cm in diameter. The purpose of using these sets of criteria is to maximize the chance of success during the transplantation. However, the use of only tumor size and number is not optimal and recent research is showing that the criteria may be too restrictive, preventing many patients from receiving transplants. Furthermore, shortage of donor organs may cause a delay in the treatment, this could result in serious consequences and is therefore considered to be a major limitation.

Ablation: Ablation is a type of treatment that destroys liver tumors without removing them and is usually considered for patients who are unable to undergo surgery due to reasons like poor health or insufficient liver function. It is best used for small tumors that are no larger than 3 cm in diameter. Various ablation methods that are available include: Radiofrequency Ablation (RFA) which uses radiofrequency energy to heat and kill the tumors in patients with a limited size of 5 cm; Microwave Ablation (MWA) uses the energy from electromagnetic waves to transfer heat and kill the tumors that span larger regions; cryoablation or cryotherapy, which kills a tumor by freezing it rapidly; and ethanol (alcohol) ablation, also known as Percutaneous Ethanol Injection (PEI), which uses injections of concentrated alcohol to achieve damages in the tumors. These different methods give varying chances of long-term survival. A concern revolving around ablation is that ablation often destroys some of the surrounding healthy tissues, so it may not be the best option for treating tumors located near major blood vessels or major bile ducts. Nonetheless, as the technologies keep

evolving, ablation is improving to minimize such side-effects and remains to be an appealing option due to its flexibility in being capable of being a treatment for all stages of HCC.

Embolization: Embolization kills tumor cells by using tiny gelatin sponges or beads to block or reduce the blood flow to tumor cells. When the material used to achieve the blockage also delivers chemotherapy drugs to the tumor, the treatment is referred to as chemoembolization. Embolization is ideal for patients who have large unresectable tumors that cannot undergo surgery or ablation. One common form of embolization is Trans-Arterial Chemoembolization (TACE), which is the standard treatment for intermediate-stage HCC. TACE fills the tumor with a chemotherapeutic drug such as doxorubicin and epirubicin through drug-eluting beads, which serve as the carrier agent. The drug is released in a controlled fashion and can significantly increase its local concentration, thereby enhancing the antitumor efficacy. Another embolization method is Transcatheter Arterial Embolization (TAE), which blocks the artery that provides blood supply to the tumor to induce its death, but does not involve the delivery of chemotherapeutic drugs like TACE. Embolization is an option for patients that still possess adequate liver function. However, embolization poses the risk of reducing blood supply to not only the tumor cells but also the surrounding normal liver tissues, hence, it is not recommended for patients whose liver has suffered damage by diseases such as hepatitis or cirrhosis [4].

Radiotherapy: Radiation therapy targets high-energy rays or beams of intense energy at tumor sites causing them to shrink and subsequently be destroyed, thereby offering local treatment for unresectable or more advanced stages of HCC. However, like some other treatments mentioned above, radiation could potentially affect nearby tissues and is therefore not recommended to patients whose liver has been damaged by diseases. Intensity-Modulated Radiotherapy (IMRT), which manipulates photon and proton beams of radiation, is currently the most attractive radiotherapy for HCC as it utilizes more advanced technology to conform precisely to the target, enabling increased dosage of radiation and lowering the risk of affecting the normal tissues. Other examples of radiotherapy include RFA, which was introduced in the previous section.

Targeted drug therapy: Targeted drug therapy focuses on targeting specific abnormalities, such as genetic mutations that are associated with tumor growth and development. Past research has identified several discrete signaling pathways that contribute to the development and progression of HCC. Most of these pathways are found to involve tyrosine kinases, which activate a wide range of proteins by phosphorylation to carry out downstream signal transduction of a range of growth factors. Hence, a lot of drugs used to treat HCC are Tyrosine Kinase Inhibitors (TKIs), which can help hinder tumor growth and development. Some examples include sorafenib (Nexavar), a multikinase inhibitor that blocks several kinases critical to both tumor proliferation and angiogenesis (the formation of new blood vessels which play a major role in the development of tumors, which require a blood supply to grow larger). Other drugs such as lenvatinib (Lenvima), regorafenib (Stivarga) and cabozantinib (Cabometyx), are also multikinase inhibitors that achieve similar outcomes. Although these inhibitors can slow down HCC progression, several limitations or challenges remain. Drug resistance can be caused by random mutations in the target receptors; the multikinase inhibitors can cause off-target toxicity; and the high heterogeneity in HCC tumor cells can also make the TKIs less effective due to their specificity of targets. During the initial stages of administration, these drugs are usually effective, but most patients will eventually develop drug tolerance. To add, existing TKIs can lead to severe adverse effects such as hypertension and diarrhea, especially when a high dosage is required to treat HCC [5].

Immunotherapy: Immunotherapy uses an individual's own immune system to fight cancer. Sometimes, cancer cells can express checkpoints to avoid being eliminated by the immune cells. For instance, programmed cell Death Protein 1 (PD-1) is a cell surface protein found on immune cells that kill tumors. When PD-1 binds its ligand, PD-L1, on target cells, the immune response is inhibited. Many tumor cells can upregulate their expression of PD-L1 and through interactions with PD-1, they can escape the immune response. Drugs that help block such interactions are referred to as checkpoint inhibitors and are used as second-line treatment for HCC in patients with tumors that showed no improvements after treatment with first-line drugs like sorafenib. Some commonly used PD-1 inhibitors are nivolumab (Opdivo) and pembrolizumab (Keytruda). There are drugs that target other checkpoints like CTLA-4 as well. Similar to targeted drug therapy, patients can develop resistance to these checkpoint inhibitors in immunotherapy. In fact, approximately 85% of HCC patients do not respond to these inhibitors. One proposed solution to the lack of response from patients is combinatorial therapy, which may involve a combination of different immune checkpoint inhibitors or other drugs that also hinder tumor progression. However, combination therapy can cause significant adverse effects. These effects may be rare, but the risk of them remains to be a concern for physicians and patients.

A wide range of treatment options are available for curing or slowing the progression of HCC, and many have a high rate of success. That said, many have limitations and may not be suitable for a lot of patients. Recurrence and unintentional damage to tissues surrounding the site of treatment continue to be a concern, which calls for new treatment approaches. In this paper, we investigate the potential of probiotics in alleviating HCC, through the modulation of the gut microbiome. Details will be discussed further in the following sections [6].

Gut microbiome and connection to HCC

The gut microbiome is comprised of a diverse range of symbiotic microorganisms colonizing the gastrointestinal tract, collectively referred to as the gut microbiome, which is responsible for maintaining metabolic homeostasis and immune balance as well as protecting the human host against pathogens. Despite the vast array of direct benefits that the gut microbiome provides to the host, it can also be a cause for the development of disease. There has been mounting evidence suggesting the contribution of the gut microbiome in the development of local and distant cancers in humans and animal models. Research

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shows that future studies on the functional interaction between the gut microbiota and the host will be critical to the understanding of the role that the microbiome plays in human homeostasis and disease pathogenesis. Local bacterial-induced carcinogenesis can be exemplified by the development of gastric cancer by the bacterial species *Helicobacter pylori via* its secretion of the virulence factor toxin cytotoxin associated gene A. In contrast to local carcinogenesis, distal bacterial-induced carcinogenesis can take place in extraintestinal organs such as the liver, heart, pancreas, lungs, and brain. The liver specifically is an organ that is located extremely close and connected to the gut *via* the hepatic portal vein, which allows biologically active gut bacteria and metabolic products to be directly transferred. A plethora of molecular compounds such as bile acids, lipopolysaccharides, choline, indole derivatives and Short-Chain Fatty Acids (SCFAs) are transported to the liver. Increasing studies show that this gut-liver axis influences the progression of liver cancer, inflammation, fibrosis and cirrhosis. For instance, the high alcohol-producing bacteria *Klebsiella pneumoniae* has been implicated in the pathogenesis of non-alcoholic fatty liver disease in human patients. The gut microbiota impacts liver cancer by modulating different factors, including bile acids, immune checkpoint inhibitors and Toll-Like Receptors (TLRS). Additionally, the intestinal epithelium is crucial to maintaining epithelial integrity and is responsible for protecting the individual from the external environment by acting as a barrier. This barrier contains important antimicrobial peptides that help defend one against harmful pathogens. Tight junctions play a pivotal role in maintaining the integrity of the intestinal barrier. When disrupted, it can lead to excess gut permeability and an access point for bacterial toxins and metabolites to reach the liver *via* the gut-liver axis. Thus, a multitude of factors that stem from the gut microbiome can lead to the progression of HCC [7].

Role of SCFAs on HCC progression: SCFAs are the main metabolites produced by the gut microbiota through the anaerobic fermentation of indigestible polysaccharides such as dietary fiber and resistant starch. The most abundant (≥ 95%) SCFAs are acetate, propionate and butyrate, which are present in an approximate molar ratio of 60:20:20 in the colon and stool. These SCFAs are the energy source to colon cells and they inhibit the formation of secondary bile acids while promoting the apoptosis of cancer cells. A growing number of studies suggest that SCFAs exert crucial physiological effects on several organs, including the liver. For one, they can improve glucose tolerance by triggering the secretion of gut hormones such as GLP-1 by enteroendocrine cells. Effects of GLP1 on liver cells are evident in the metabolism of saccharides, where GLP1 stimulates the synthesis of glycogen and reduces glucose production. Additionally, SCFAs are directly connected to the liver through the hepatic portal circulation where they also influence glucose metabolism. In an experiment conducted by Besten and colleagues, 62% of infused propionate was involved in the production of glucose. Results of the study highlighted that upon SCFA treatment, there was more efficient glucose uptake with lower demand for insulin. Additionally, SCAFA's role in glucose uptake is crucial for liver cancer patients because tumor cells lose the ability to produce and release glucose into the bloodstream. An increase in levels of SCFAs in the gut could improve liver function and possibly induce additional effects to slow liver cancer progression. Among the three main SCFAs, butyrate has the potential of behaving as an antitumor metabolite. Butyrate has an apparent Histone Deacetylase (HDAC) inhibitory property which helps to combat tumor progression. This property of butyrate enables the inhibition of cell proliferation and also induces apoptosis, which are desirable mechanisms for preventing the growth of abnormal cells. It is also worth noting that butyrate plays a crucial role in increasing tight junction proteins to reduce gut permeability. This would prevent bacterial toxins in the gut from being released into the liver. In liver cancer patients, the hepatic concentration of butyrate is very low. According to a study by Sheng et al. human HCC specimens had reduced SCFA receptor genes which could be correlated with reduced butyrate production. The study also suggested that increasing butyrate levels may influence the concentration of acetate and propionate, which in turn would positively affect liver health and function [8].

Role of bile acids on HCC progression: Bile Acids (BAs) are another large family of molecules that can advance the progression of liver cancer. BAs can be classified into primary and secondary BAs according to their metabolic process. Primary BAs are synthesized in hepatocytes from cholesterol and are reabsorbed in the distal ileum. However, 5% to 10% of BAs that are not reabsorbed in the distal ileum and escape enterohepatic circulation (200 mg-800 mg daily in humans) undergo biotransformation to form secondary BAs. The main bile salt conversions in the human gut lead to the presence of over 20 different secondary BAs in adult human species. These secondary BAs play an important role in glucose metabolism as well as vitamin and lipid absorption. However, when BAs are in excess, they can cause hepatocyte DNA damage and promote carcinogenesis by altering tumor suppressor genes and inducing stress response signaling. High levels of BAs in the liver can also cause a liver disease known as cholestasis, which was found to be linked with liver cancer in several clinical studies. Secondary BAs, particularly Deoxycholic Acid (DCA), are known to activate a number of cell-signaling pathways which are risk factors for inflammation and cancer. Epidemiologic research has demonstrated that both colon cancer patients and people eating a high-fat diet have higher levels of fecal secondary BAs, primarily DCA and LCA. A high-fat diet raises the risk of colon cancer by increasing the amount of bile acid Deoxycholic Acid (DCA) in the colon lumen. Reactive Oxygen Species (ROS), cytochrome C, mitochondrial oxidative stress and cytosolic caspases are among the intrinsic apoptotic mechanisms that are primarily activated by elevated colonic DCA concentrations. Consequently, some colonic epithelial cells may develop resistance to the apoptosis caused by BA and the development of colon cancer has been associated with this cell subpopulation. Reducing excess amounts of BAs in liver cancer patients may help re-establish homeostasis and prevent the progression of cancer to advanced stages.

Role of toll-like receptor expression on HCC progression: In addition to DCA production, Toll-Like Receptors (TLRs) could also be an indicator of liver cancer progression. As part of the innate immune system, TLRs recognize pathogen-associated molecular patterns in various microbes and initiate an innate immune

response. In liver cancer, TLR4 and TLR9 specifically play an important role in the liver-inflammation-fibrosis-cancer axis. When changes in the gut microbiota lead to reduced integrity of the intestinal barrier, increased leakage of lipopolysaccharides and fatty acids act upon TLR4 to activate systemic inflammation, leading to liver injury and thus, promoting HCC. Preclinical studies with mice models have shown that the activation of the TLR4 signaling pathway in response to the toxic effects of secondary bile acids also promotes HCC development. However, reducing the expression of these TLRs, especially TLR4 and TLR9, can help regulate immune responses that lead to the progression of HCC. In recent years, there has been a rapid rise in interest in using probiotics to reduce TLR expression, reduce excess amounts of BA, and increase SCFA levels [9].

Introduction to probiotics

Probiotics: Probiotics are defined as live, non-pathogenic microorganisms, such as bacteria and yeast, that are generally regarded as safe and confer health benefits when consumed or administered to an individual in adequate amounts. They may help produce substances that are beneficial to one's health, influence an individual's immune response and help to form and sustain a healthy community of microorganisms. Typical sources of probiotics include yogurt, fermented foods and dietary supplements. The most common probiotics are bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium*. It is important to note that distinct probiotic strains may elicit varied effects. For instance, a particular strain of *Lactobacillus* may exhibit an efficacy in preventing a certain illness but this does not necessarily mean that another *Lactobacillus* strain will have the same success in preventing that illness. In other words, the selection of probiotic strains must be highly specific as outcomes are strain-dependent and not universally applicable across all strains.

Probiotic mechanism of action: Probiotics provide health benefits through several major mechanisms including maintenance of the intestinal epithelial barrier, competition for adhesion sites with pathogenic microorganisms, production of antimicrobial metabolites and modulation of the immune system.

The intestinal epithelium functions as a protective barrier to inhibit the penetration of harmful substances, such as bacteria and endotoxins, into the intestinal wall and therefore prevent their entry into tissues, organs and microcirculation. Tight junctions, located between intestinal epithelial cells, uphold this function by selectively transporting beneficial substances, and inhibiting the entry of pathogenic bacterial and harmful substances, into the intestinal lumen. Hence, tight junction damage may lead to "leaky gut syndrome" whereby pathogens or potentially harmful substances obtained from the diet reach the deeper layers, such as the submucosa, and trigger inflammatory responses resulting in intestinal disorders such as inflammatory bowel diseases. In cases of tight junction disruption or damage, consumption of probiotics can restore the integrity of the intestinal barrier through increased expression of genes and proteins involved in tight junction signaling and through modulation of apoptosis and proliferation of intestinal epithelial cells [10].

Another mechanism through which probiotics provide protection against enteropathogens is through their adhesion to the intestinal mucosa. In doing so, probiotics compete with enteropathogens for host cell binding sites and therefore reduce enteropathogen colonization in the gut. Moreover, the ability of probiotic bacteria to adhere to intestinal mucosa could lead to prolonged transit time in the gut, allowing them to carry out their intended positive effects. Adhesion of pathogenic bacteria to mucosal surfaces is usually the first step of intestinal infections.

Furthermore, probiotics can produce helpful substances including organic acids and other compounds with antimicrobial activities. Organic acids, such as acetic acid and lactic acid, have strong inhibitory effects against gram-negative bacteria and are regarded as the main antimicrobial compounds that grant probiotics their inhibitory activity against other pathogens. These organic acids, in their undissociated forms, can enter the bacterial cell, dissociate inside its cytoplasm, lower the pH and/or accumulate and eventually result in the death of the pathogens. Apart from organic acids, probiotics can also produce antibacterial peptides termed bacteriocins, which usually kill their target cells with high potency by forming pores in their walls and/or inhibiting their cell wall synthesis [11].

In addition, probiotics can benefit health through modulation of the host's innate and adaptive immune response by modulating dendritic cells, macrophages and B and T lymphocytes. Probiotics upregulate the expression of genes that stimulate cytokine production which in turn activates T regulatory cells. T regulatory cells act to suppress the immune response which maintains homeostasis and self-tolerance; playing a critical role in preventing autoimmune diseases and inflammatory immune disorders. Moreover, probiotics may enhance the intestinal immune function by stimulating B cells to produce IgA which has a crucial role in protection against harmful antigens.

Probiotics as a solution for HCC: Given that dysbiosis of the gut microbiome implicates the risk of liver cancer progression, probiotics and their metabolic products are being proposed as a potential solution to mitigate or prevent the risk of liver tumorigenesis. According to studies with liver disease patients, probiotics may help populate the gut microbiome with beneficial bacteria that can strengthen intestinal barrier function and tight junction integrity to prevent toxic substances from being released into the liver. Additionally, several bacterial strains have been shown to produce anti-inflammatory metabolites, such as Short-Chain Fatty Acids (SCFAs), that can reduce carcinogenesis by inhibiting cell growth and migration, suppressing histone deacetylase and inducing apoptosis. The *Firmicutes* phylum is a classification of bacteria that produces the anticarcinogenic primary metabolic product butyrate, proven to inhibit the growth of HCC cells. Under normal physiological conditions, the majority of gut microbiota consists of microorganisms from the *Firmicutes* phylum whereas acetate and propionate are mainly produced by the *Bacteroides* phylum. Additionally, probiotics may contribute to the deconjugation of

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BAs, which make BAs insoluble and thereby lower the aqueous concentration of BAs and thus reducing their tumor-progressing effect on liver cancer. Lastly, there is some evidence that suggests that probiotics can inhibit the TLR4 and TLR9 activation pathways to reduce inflammation and HCC progression, although the studies remain limited [12].

Traditional probiotic bacteria candidates for cancer applications

Numerous preclinical and clinical trials have been conducted to help investigate the effects of probiotics on cancer treatment, by focusing on modulating the gut microbiota composition. It is important to note that some trials have reported an improved clinical outcome in cancer patients who have received probiotics, whereas others have failed to demonstrate significant effects induced by probiotic administration. The results of many *in vitro* studies have shown that probiotics have a multitude of beneficial properties in regulating the proliferation and apoptosis of cancer cells. Currently, most of the research being conducted on probiotics and cancer treatment is on gastrointestinal tumors. Specifically, a prospective intervention study on patients with Colorectal Cancer (CRC) demonstrated that the administration of *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bl-04 modified the patients' microbial profile. These probiotics increased the abundance of butyrate-producing bacteria such as *Faecalibacterium* while decreasing the prevalence of CRC-associated bacterium, such as *Fusobacterium* and *Pepto streptococcus*. CRC patients have insufficient levels of butyrate in their stools and thus, patients are unable to inhibit the development of CRC. Consumption of probiotics such as *Lactobacillus acidophilus* helps increase the daily production of SCFAs, which allows for the inhibition of carcinogenic pathogens. A recent research study was conducted on an azoxymethane-induced CRC mice model treated with a probiotic mix composed of 7 different strains of lactobacilli, bifidobacteria and streptococcus, which exhibited suppression of colon carcinogenesis due to modulation of mucosal CD4+ T polarization and alteration in gene expression. A follow-up experiment investigated the effects of *B. infantis* administration in a CRC rat model, which correlated with decreased levels of proinflammatory cytokines and increased levels of the CD4+ and CD25+ T regulatory cell response. It has been found that patients with colorectal cancer usually have a greater proportion of bacteria that cause gastrointestinal inflammatory diseases, which can produce carcinogenic toxins. Studies have shown that chronic inflammation can make individuals susceptible to cancer. A study that was conducted found that under the mucus layer of the colon, a species known as *Clostridium* spp. were in direct contact with colon cells, causing persistent local inflammation. This strain has been found to be abundant in CRC patients, and has exhibited a profile of inflammation-related genes and proteins including COX-2, NF-κB, TNF-α, IL-6, IL-8, and IL-12 which contribute to tumor occurrence. A group of researchers utilized a CRC animal model and found that the use of the probiotics *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* or both in combination resulted in reduced NF-κB, COX-2, β-catenin and K-ras carcinogenic biomarkers [13].

In addition to gastrointestinal tumors, abnormal changes in the composition and function of the gut microbiome could also affect non-gastrointestinal tumors, such as pancreatic cancer, liver cancer, breast cancer and lung cancer. For example, a recent study has found that the probiotic *Lactobacillus rhamnosus* can boost the ability of immune cells to target and eliminate lung cancer cells. When the probiotic was utilized synergistically with the chemotherapeutic drug dacarbazine, the treatment efficacy significantly improved in high-risk melanoma patients. In conclusion, preclinical and clinical studies have found that probiotics induce tumor cell apoptosis and inhibit cancer cell proliferation. However, it is important to note that most current research on probiotics and cancer focuses on gastrointestinal tumors and the specific mechanism of probiotics against tumors has not yet been fully elucidated. Therefore, the therapeutic effects of probiotic application on cancer are currently still under rigorous investigation. As previously mentioned, probiotics have been used to treat a plethora of other cancers and have a strong potential to also improve liver cancer outcomes for HCC patients. Since probiotic application is well studied for gastrointestinal tumors, this information can be extrapolated and applied in the development of probiotic therapeutics for HCC.

Potential probiotic bacteria candidates for liver cancer treatment

Bile acid-reducing bacteria: *Lactobacillus* strains are popular probiotic candidates that have demonstrated beneficial health effects in numerous diseases. Multiple studies have examined the potential of *Lactobacillus* strains in reducing BA production which was described previously as one of the major risk factors of HCC progression. A study by Zhang et al. investigated the effect of *Lactobacillus casei* on fecal BAs and inflammation in rats with type 2 diabetes. The study found that compared to a simple High-Fat Sucrose (HFS) diet, secondary BA levels were significantly lower in a combined diet of *L. casei* and HFS. Likewise, data from qPCR quantification revealed a significant increase in a major BA producing species called *Clostridium scindens* in the HFS diet relative to the combined HFS+ probiotic diet. This could indicate that the *L. casei* probiotic may either reduce *C. scindens* levels or the excess BA produced by *C. scindens*. Additionally, another randomized, double-blind, placebo-controlled study was conducted in cirrhotic patients to study the influence of an alternative *Lactobacillus* probiotic called *Lactobacillus* GG (LGG). In the presence of LGG, the study demonstrated a significant increase in several members of the *Firmicutes phylum* such as *Lachnospiceae* and *Clostridiales* XIV, which are gram-positive bacteria associated with lowering the risk of diseases. Conversely, the study reported a decrease in levels of *Enterobacteriaceae* and *Porphyromonadaceae* which are associated with disease progression. The results of the study were compared to the placebo group where increased levels of DCA had been observed in absence of the probiotic. Given that *Lactobacillus* strains can reduce BA levels, their use as probiotics to treat liver cancer should be investigated further to optimize treatment strategies [14].

Butyrate-producing bacteria: Over the last decade, new probiotic candidates demonstrating potential health benefits have gained increasing recognition. These candidates mainly belong to butyrate-producing members of *Clostridium* clusters IV and XIVa (e.g. *Faecalibacterium prausnitzii*) or to the health-promoting mucin degraders *Akkermansia muciniphila*. A recent study examined the activity of butyrate-producing genera on the gut microbiome in patients with cirrhotic Hepatitis B Virus (HBV)-HCC and HBV-cirrhosis. The study observed decreased levels of several butyrate-producing genera namely *Ruminoccocus*, *Oscillibacter*, *Faecalibacterium*, *Clostridium* IV and *Coprococcus* in HCC patients compared to other conditions. These butyrate-producing genera were associated with energy metabolism, intestinal homeostasis and immune response regulation. Another study analyzed the pattern of dysbacteriosis in HCC caused by different etiologies. The study observed a decrease in bacteria belonging to the *Firmicutes* phylum suggesting a potential role of bacteria in this phylum in regulating liver health.

F. prausnitzii is a species of bacteria that belongs to the *Firmicutes* phylum as a member of the *Ruminococcaceae* family. It is one of the most abundant bacterial species in the colon where it represents 5%-15% of all gut bacteria. *F. prausnitzii* has recently gained prominence as a crucial bioindicator of human intestinal health. A number of studies have noted a decreased abundance of this bacterium in several Inflammatory Bowel Diseases (IBD) such as CD and Ulcerative Colitis (UC), as well as CRC and type 2 diabetes. The potential of *F. prausnitzii* as a probiotic has been discussed in several studies to restore dysbiosis from IBDs. The health benefits of *F. prausnitzii* originate from its ability to produce large amounts of butyrate and consume acetate in the colon which favorably modulates the intestinal immune system. Numerous studies have found that *F. prausnitzii* levels in the human intestinal system negatively correlate with levels of TLR4. A recent study by Zhang et al. has outlined the mechanism by which *F. prausnitzii* provides anti-inflammatory effects by downregulating the expression of TLR4 in colon epithelium. This demonstrates the potential of *F. prausnitzii* in modulating TLRs associated with liver cancer progression. Another study by Xu et al. found that *F. prausnitzii* could restore the intestinal barrier structure and function *via* the regulation of the tight junction pathway. This process is aided by a recently discovered metabolite of *F. prausnitzii* known as the Microbial Anti-inflammatory Molecule (MAM). In a mice model, MAM was shown to reduce the colonic epithelial intercellular gap, which would prevent pathogens and other potentially harmful substances from entering the liver [15].

Although *F. prausnitzii* is a very abundant bacterium in the colon, their basic research and clinical application as a probiotic remain limited. One major reason is their extreme sensitivity to oxygen. They are considered strict anaerobes that lose viability within two minutes after exposure to ambient air. This makes it extremely difficult to cultivate the strain in synthetic media and be used to restore dysbiosis in liver cancer patients. Another reason the cultivation of *F. prausnitzii* is difficult is due to their specific nutritional requirements. Recently, several studies have come up with solutions to keep the obligate anaerobe viable in ambient air by adding specific antioxidants. For example, several *in vitro* monocultures have shown that strict anaerobes grow well in media supplemented with inulin, riboflavin and L-cysteine antioxidants. To optimize specific growth conditions for *F. prausnitzii*, sugars such as fructose and fructo-oligosaccharides can also be added to the media to maximize the cultivation of *F. prausnitzii*. Further investigation is required to obtain *F. prausnitzii* in large amounts so that its influence on liver cancer can be tested and the product can be distributed to a larger number of liver cancer patients [16].

Discussion

It can be seen that the gut-liver axis, *via* the connection of the hepatic portal vein, plays an important role in the pathogenesis of liver diseases such as HCC. Through the expansion of growing evidence that has supported the role of the gut microbiota in the development of HCC, it can be determined that the manipulation of the gut microbiome *via* the application of probiotics serves as a potential novel therapeutic intervention to treat HCC. The application of probiotics represents a cutting-edge, safe and low-cost strategy to treat HCC. However, more laboratory-based mechanistic studies, as well as extensive human clinical trials in the evaluation of potential probiotics for HCC, are essential to gain acceptance of the broader medical community for the use of probiotics as a novel therapeutic intervention for liver cancer. Currently, the majority of clinical studies associated with the use of probiotics are for gastrointestinal tract illnesses such as travelers' diarrhea. Despite this, it is still unclear whether or not other illnesses that are not related to the gastrointestinal tract could benefit from the use of probiotics [17].

Currently, there is a clinical research study under critical investigation regarding the application of probiotics as a therapeutic intervention for liver cancer that is in the process of presently recruiting participants with an estimated enrollment of 46 individuals who are between the ages of 18-70 of all genders and diagnosed with liver cancer. The first research study examines how probiotics enhance the treatment of PD-1 inhibitors in patients with liver cancer. Programmed Death receptor 1 (PD-1) is a critical immunosuppressive molecule that regulates the immune system and promotes tolerance by down-regulating the immune system's response to human cells and by suppressing T-cell inflammatory activity. In the past, this research team has found strong correlations between gut microbiome efficacy and anti-PD-1 immunotherapy in cancer patients. This current study is taking place in Jiangxi Provincial Cancer Hospital, in Beijing, China and is projected to be completed by December 30, 2023. The experimental design consists of patients who are assigned into groups based on randomized allocation using a computer-generated random number code. The patients were also randomly distributed in a 1:1 ratio to receive either the probiotics treatment or the placebo. The probiotics experimental group received the oral probiotic *Lactobacillus rhamnosus* which was administered one time a day during the entire duration of the treatment. The experimental outcomes would be quantitatively measured by

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examining the proportion of patients with tumor volume shrinks to a predetermined value [18].

There has also been a relatively recent study that was completed on April 12th, 2018, that examined the influence of probiotic administration before liver resection in liver disease. The study design consisted of 64 participants who were of all sexes from the ages of 18 years and older. The inclusion criteria consisted of a patient with a confirmed diagnosis of HCC, liver disease, indication of surgical resection, a patient that is operable and has a resectable tumor lesion. As previously mentioned, surgical resection is one of the curative treatment modalities for HCC. Some of the limitations are postoperative septic and liver functional complications related to an increase in bacterial translocation and endotoxemia. Bacterial translocation results in the passage of bacterial degradation products from the intestine to the hepatic portal circulation, ultimately leading to nitric oxide production causing membrane instability of vascular endothelium and intestinal mucosa. The study proposed the utilization of probiotics to potentially improve liver function and decrease infectious complications in patients with chronic liver disease and HCC. The participants were assigned through random assortment into the respective experimental and placebo groups. The experimental group received an oral 560 mg probiotics capsule that is composed of a mixture of 10% *Bifidobacterium lactis*, 10% *Lactobacillus acidophilus*, 40% *Lactobacillus plantarum*, 20% *Lactobacillus salivarius*, 20% *Bifidobacterium lactis* 304 dosage. The primary outcomes of the experiment that were measured were relative levels of circulating plasma endotoxin concentrations over time [19].

Despite numerous data supporting the anti-tumor activity of probiotics, experimenting with a larger range of unique probiotic strains is required in comparative studies to identify which strains are most effective for treating liver cancer. Additionally, there appears to be a lack of conclusive evidence on the role of probiotics in human liver cancer patients given the small sample size of liver cancer patients used in currently existing studies. Thus, more clinical studies with liver cancer patients are needed to be tested. Additionally, the probiotic strains that are currently in the market are limited to mainly *Lactobacillus* strains, requiring further testing to be done with novel strains like *F. prausnitzii* for future preclinical trials [20].

Conclusion

Currently, there are effective treatment options for liver cancer that may be able to cure some people of the disease or reduce its progression. Given that treatment for liver cancer normally only begins once symptoms are present, regular intake of probiotics once gut dysbiosis is detected could be a preventive measure for carcinogenesis, and could be combined with current drug treatments to improve the efficacy of eliminating the disease. While probiotics may not be a cure, their benefits are multifaceted. The proposed therapy takes an approach that is largely dependent on what is already known, thus seeming to be theoretically feasible.

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